



NASA ASTROBIOLOGY INSTITUTE ANNUAL REPORT YEAR [July 2003 - June 2004]



Annual Reports :: Year 6 :: Indiana University

Team Reports: Indiana University

Indiana University
Executive Summary
Principal Investigator: Lisa Pratt

Introduction. During the first six months of funding from the NASA Astrobiology Institute (NAI), three major research projects were initiated by the Indiana–Princeton–Tennessee Astrobiology Initiative (IPTAI) Team: 1. Geomicrobiology and hydrogeochemistry of intra– and sub–permafrost water intersections in a deep gold mine, Kinross Luipn Mine, Nunavat Territories, Canada; 2. Partitioning of sulfur isotopes during pyrite oxidation coupled to radiolytic water cleavage; and 3. Whole–genome sequencing of an uncultured *Desulfotomaculum*–like organism from hydrothermal waters in deep gold mines, Witwatersrand basin, South Africa. Each of these projects requires a high degree of collaboration among IPTAI laboratories and involves two to four principal investigators, one to three post–doctoral associates, and two to five graduate and undergraduate students.

The central focus of the IPTAI team is investigation of psychrophilic microbial communities in a deep gold mine located near the Arctic Circle in northern Canada.



Figure 1. Headframe and living quarters (red buildings) at Kinross Lupin Gold Mine, Nunavut Territories, Canada. View of the mine from the winter haul road in May 2004.

This project involves highly instrumented field and laboratory activities (Figure 2) that build on expertise developed during 5 years of work on thermophilic microbial communities in the deep and ultra-deep gold mines of South Africa. Intra-permafrost/sub-permafrost brines from Arctic and Antarctic regions are important terrestrial analogues for Martian groundwater. Delineating biogenic signatures in analogue environments is crucial for designing life-detection probes for deployment on future Mars exploration missions.



Figure 2. Tullis Onstott (IPTAI–Princeton) and Monique Hobbs (Ontario Power Generation) working to install a noble gas diffusion sampler at the 890 meter level, Lupin Mine.

1. Geomicrobiology and hydrogeochemistry of intra- and sub-permafrost water intersections in a deep gold mine, Nunavut Territories, Canada. PIs Onstott, Pratt, Sherwood–Lollar, Clifford, and Pfiffner. Distinct microbial communities are anticipated in brines associated with continuous permafrost but uncontaminated samples of permafrost brines are rarely available for scientific study. The main shaft, ore workings, and exploration drifts at Lupin mine allow controlled study of both intra- and sub-permafrost waters. Water intersections at Lupin are located in fracture zones hosted by low-permeability Archean metagraywacke, phyllite, and banded iron formation. An initial field trip to assess the scientific potential at Lupin was conducted in May 2004 when Pratt and Onstott from IPTAI and Corien Bakermans from Michigan State University (MSU) joined an international team of permafrost investigators for collection of water and rock samples (Figures 3 and 4). A second sampling trip is planned for fall 2004. Samples from the May 2004 field trip will be given to the Marine Biological Laboratory (MBL) for assessment of eukaryotic activity. It is particularly exciting to link IPTAI's deep-subsurface expertise with MSU's resources as a center for research on psychrophilic microorganisms from Siberian permafrost and Antarctic lake ice and MBL's resources as a center for research on extremophile eukaryotes.



Figure 3. Tullis Onstott (IPTAI–Princeton) inoculating iron-reducing media with subsurface brine at the 1130 meter level, Lupin Mine.



Figure 4. Corien Bakermans (MSU lead team) injecting subsurface brine into a sterile serum vial filled with nitrogen, Lupin Mine.

With the assistance of Timo Ruskeeniemi (Geological Survey of Finland) and Monique Hobbs (Ontario Power Generation), brines of widely varying salinity were collected from 11 subsurface sites in May 2004. Brines below the permafrost were collected from six drill holes outfitted with valves and pressure gauges and located at the 1130– and 880–m levels. Dripping water from open fractures in the roof was collected at the 1130– and 250–m levels. Dripping water at the 250–m level is within the current permafrost. Water recirculated within the mine for drilling activities (service water) was sampled from an open drain at the 1130–m level. The following types of aliquots were collected:

- a. Filters for DNA analyses.
- b. Filters for enrichment of cultivable, anaerobic and aerobic psychrophiles.
- c. Anaerobic media (sulfate reducers, fermenters, Fe (III) reducers and

methanogens) inoculated with borehole water.

d. Gas samples for isotopic analyses and measurement of dissolved H_2 and CO .

e. Water sample for isotopic analyses, including N isotopes of NH_4^+ (Waterloo)

f. Noble gas samples (Ottawa).

g. Dissolved sulfate/sulfide samples for isotopic analyses.

h. Samples for FISH (Fluorescent In Situ Hybridization) and flow cytometer (cell density)

i. Multiple samples for analyses of cations, anions, and short-chain fatty acids.

Field parameters, pH, Eh, dissolved O_2 , temperature and conductivity were measured on site. Conductivity ranged from 4.9 to 60.4 mS/cm. Temperatures varied from 13.4 to 15.5°C. The pH ranged from 7 to 9. Visible ZnS precipitates were observed in the sulfur isotope syringes after one day of storage at room temperature indicating the presence of significant sulfide concentrations. Although Eh values as low as -143 mV were measured, Eh values were higher than expected for a sulfate/sulfide redox couple at the observed pH and temperature. One possible explanation is that mixing of water with different reduction potentials from separate fractures is occurring within boreholes. Televue logs indicate the presence of multiple fractures, and fracture water chemistry seems highly variable based upon observations of seeps.

The integrity of borehole installations at Lupin is a tribute to the technical ability of the Finish/Canadian collaborators on the Permafrost at Lupin Project. We know of no equivalent borehole array available for scientific study at a deep mine. Of particular interest for sampling deep subsurface microbes, there are high *in situ* water pressures (300 to 700 psi), sulfide is present, dissolved O_2 is absent, and the boreholes have been isolated for time periods up to 14 months. These are important positive indicators that the borehole microbial communities represent indigenous organisms in the rock formation. Over the next several months, the sulfur isotopic and DNA analyses combined with the results of microbial enrichments will allow assessment of the extent of potential mining contamination of the environment and to target specific boreholes for more detailed sampling via a borehole packer system.

2. Partitioning of sulfur isotopes during pyrite oxidation coupled to radiolytic water cleavage. PI's Ripley, Sherwood-Lollar, Pratt, and Onstott. A series of experiments are designed to investigate sulfur isotopic effects that may accompany the oxidation of sulfide minerals initiated by reaction with products formed during the radiolysis of water molecules. Radiolysis produces elevated H_2 concentrations in waters, as well as strong oxidants such as H_2O_2 and O_2 . Both H_2O_2 and O_2 may react with sulfide minerals as S^0 , $S_2O_3^{2-}$, or SO_4^{2-} . Thiosulfate ($S_2O_3^{2-}$) can undergo disproportionation to produce both oxidized (SO_4^{2-}) and a reduced (S^{2-}) sulfur species. Because of the isotopic fractionation between reduced and oxidized species, large δ (sulfate-sulfide) values may be a result of radiolytic oxidation processes. Sulfate-reducing bacteria could utilize the oxidized sulfur species with the ultimate production of sulfide minerals. However, the generation of sulfide minerals from the reduced species liberated from thiosulfate could be characterized by $\delta^{34}S$ values that are similar to those produced in sulfide minerals where the reduced sulfur is of biologic origin. We must understand the

potential sulfur isotopic effects that are associated with each of these processes.

To this end we have been involved in designing a methodology to recover both reduced and oxidized sulfur species from thiosulfate. To date, 32 experiments to evaluate the sulfur isotopic mass balance in the disproportionation of $\text{Na}_2\text{S}_2\text{O}_3$ have been completed. A thiosulfate solution is formed by mixing powdered sodium thiosulfate ($\text{Na}_2\text{S}_2\text{O}_3$) with deoxygenated, nanopure water under a N_2 atmosphere. The thiosulfate solution has been reacted with silver nitrate (AgNO_3) for periods varying from 1 to 2 hours; this step leads to the recovery of reduced sulfur as Ag_2S . After the removal of Ag_2S (Figure 5) by filtration, the residual solution is heated to 80°C and $\text{Ba}(\text{NO}_3)_2$ is added to produce BaSO_4 . Initial experiments utilized BaCl_2 , but excessive amounts of AgCl were formed and prevented a quantitative recovery of BaSO_4 . Isotopic measurements are in progress to determine the most efficient protocol to accurately characterize the distribution and isotopic fractionation of sulfur species. This work constitutes a portion of the Ph.D. research of Irene Arango at Indiana University.

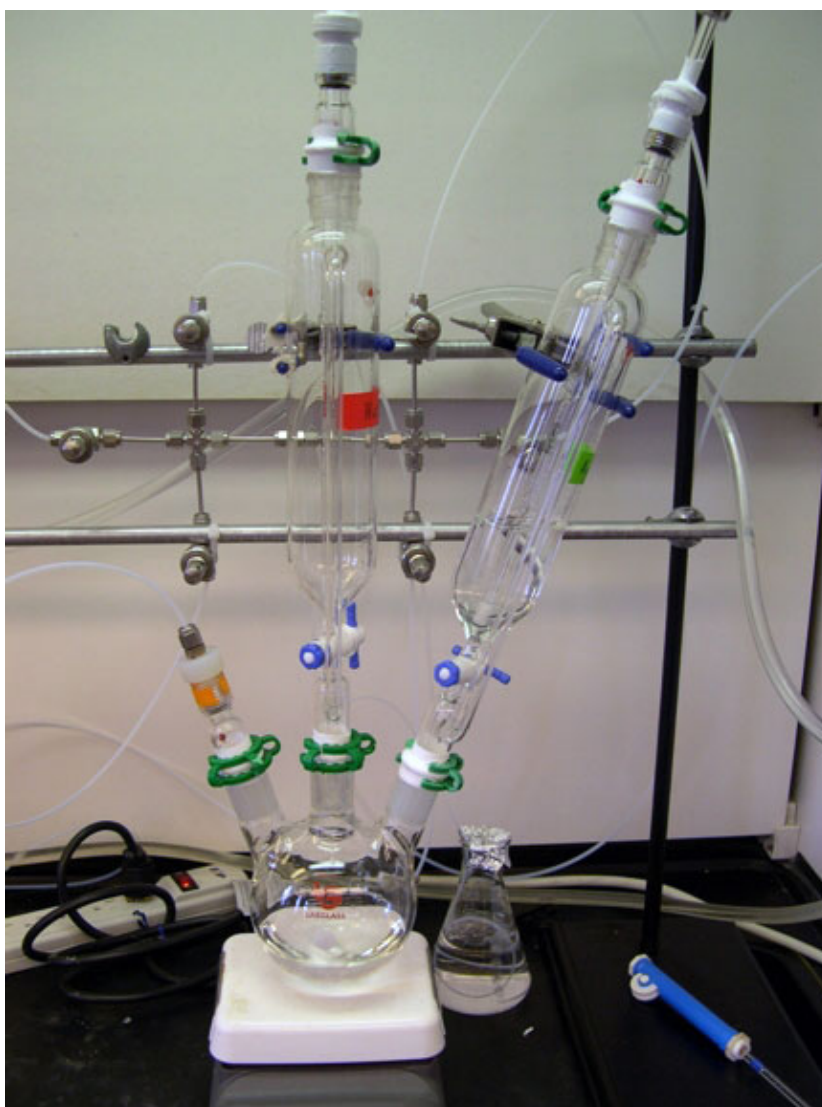


Figure 5. Nitrogen-purged extraction apparatus for quantitative recovery of dissolved and volatile sulfur species, Biogeochemical Laboratories, Indiana University.

In addition to the experiments on thiosulfate and its disproportionation products we will load our first closed-system pyrite oxidation experiments in July. Hydrogen peroxide will be syringed through a septum into a Pyrex tube containing finely ground pyrite. The tube will be sealed and the experimental charge heated for a 1- to 2-week period. Various temperatures will be utilized to evaluate the kinetics of the pyrite oxidation reaction.

During the past six months, Indiana University advertised for our post-doctoral position. Several excellent candidates responded, and our evaluation and interview process was necessarily thorough. The position was offered to Ms. Lili Lefticariu who has recently completed her Ph.D. dissertation involving stable isotopic geochemistry at Northern Illinois University. Lili will be in Bloomington in early July to discuss the project and will begin her work in mid-August. Over the coming year, experiments running at Indiana will be coordinated with similar experiments running at Toronto in order to study

carbon isotope effects on oxidized and reduced carbon species.

3. Whole-genome sequencing of an uncultured *Desulfotomaculum*-like organism (DLO) from hydrothermal waters in deep gold mines, Witwatersrand basin, South Africa. PI's Brockman, Hazen, and Onstott. A deep-branching clade of nearly identical DLO sequences (>99% homology) has been identified in 6 boreholes in 4 mining complexes separated by as much as several hundred km. The DLO has been the dominant bacterium (>90–100% of clones) present in clone libraries from very high quality sample sets in two different mines, and their dominance was retained throughout a several month time series in one case, and a 648-m vertical depth profile in the other case. Sulfate reduction appears to be the dominant terminal electron accepting process in these sample sets based on sulfate and sulfide levels, sulfur isotope geochemistry, and Gibbs free energy calculations. While prediction of physiology from phylogeny is not straight-forward in this case, the environmental chemistry, the DLO's strong dominance in the community, and the DLO's affiliation with cultivated *Desulfotomaculum* spp. that are sulfate-reducers all speak to the likelihood that it is a sulfate-reducer. The closest cultured relative is *Desulfotomaculum kuznetsovii* (90% similarity) and the closest sequence in Genbank is an environmental clone recently recovered from oceanic crustal fluid (95% similarity). Multiple attempts by different laboratories to culture the DLO have been unsuccessful.

To gain an understanding of the capabilities and degree of genetic novelty of the DLO, we have filtered large quantities of fissure water from a sample dominated by the DLO in order to provide adequate biomass for community DNA sequencing. The Department of Energy Production Genomics Facility will conduct the sequencing in collaboration with Pacific Northwest National Lab and Lawrence Berkeley National Lab. A total of 35,000 liters containing 4×10^4 cells per ml were filtered through a large area filter cartridge. This filter should yield 280 micrograms of DNA assuming a genome size of 2 Mbp/cell and 10% extraction efficiency. A minimum of 10 micrograms is needed for shotgun sequencing

DNA was extracted from 15% of the filter and quantified by spectrophotometry and gel electrophoresis. DNA yield was poor with only 400 nanograms of DNA recovered from 15% of the filter, indicating an extraction efficiency of only 0.1%. In addition, only 25% of this DNA was of the size (10 kb or larger) needed for shotgun sequencing. To preserve as much filter as possible, two other extraction protocols were used (as a screen) on smaller aliquots of the filter to determine if they would produce detectable DNA. No DNA was detected with these methods. These results indicate that DNA extraction efficiency is much poorer than expected.

In an effort to optimize the extraction efficiency, 1×10^{12} *Arthrobacter* cells (a difficult to lyse Gram positive bacteria used as a model for the DLO) were filtered through an identical filter cartridge. A different extraction protocol (using lysozyme-sodium dodecyl sulfate/Proteinase K-guanidine isothiocyanate, with a liquid nitrogen and mortar and pestle grinding pretreatment) was used and compared against the previous extraction method. DNA extraction efficiencies were 11% with the new method and 0.5% with the original method. In addition,

all DNA was greater than 10 kb in size. The new protocol will be tested on a small fraction of the filter containing the DLO, and once its performance has been confirmed to be adequate, one half of the remaining filter containing the DLO will be extracted.

Education and Public Outreach (EPO). Outreach for the IPTAI Team is centered at the University of Tennessee and is under the direction of Susan Pfiffner. Funds from NAI were used to match funds from the National Science Foundation to support a seven-week Research Experience for Undergraduates (REU) held in South Africa and targeted toward minority students. The purpose of the South African REI is to engage students in geomicrobiological research and to encourage students to think about scientific careers. American students work side by side with South African students under the joint supervision of U.S. and South African faculty. One Taiwanese, ten American, and six South African students participated in the summer 2004 program. In addition to REU activities, eight lectures and seven media interviews were handled by Pratt, Onstott, and Pfiffner during the first six months of NAI funding for IPTAI . Additional information on EPO activities is available on the IPTAI website at (<http://www.indiana.edu/~deeplife>).